21A-3-5-20



Reviewer: S. Semalulu , Date April 11, 2001

**STUDY TYPE:** Multigeneration Reproduction Study - Rat. OPPTS 870.3800 (§83-4); OECD 416.

**TEST MATERIAL (PURITY):** SZX 0722 (Iprovalicarb).

**SYNONYMS:** Melody

**CITATION:** Eiben, R (1997) SZX 0722: Two-generation study in Wistar rats. Bayer AG, Institute of Toxicology, Wuppertal, Germany. Report No. 26391, (June 24, 1997). MRID not available. Unpublished

**SPONSOR:** Tomen Agro Inc.

**EXECUTIVE SUMMARY:**

In a 2-generation reproduction study (MRID not available]), SZX 0722 (99.2%) was administered to 28-30 Wistar (ICO:WU (IOPS Cpb) rats/sex/dose in the diet, at dose levels of 0, 100, 2000 or 20000 (equal to pre-mating doses: 0, 7.3, 146.3 or 1514.3 mg/kg bw/day in males and 0, 9.6, 190.4 or 2074.0 mg/kg bw/day in females of $F_0$ generation and 0, 7.7, 155.3, 1838.0 mg/kg bw/day in males or 0, 10.8, 239.5, 2944.1 mg/kg bw/day in females of F1 generation), over 2 generations with one litter per generation [mean 0, 7.5, 150.8, 1676.2 or 0, 10.1, 214.95, 2509.1 mg/kg bw/day in males and females respectively].

There were no treatment-related clinical signs, or mortality in $F_0$ or $F_1$ parental animals of either sex at all dose levels. At 20000 ppm, parental $F_0$ and $F_1$ females consumed more feed (14.7% and 23% respectively) than the controls during the pre-mating period, and $F_1$ males had significantly decreased (10%) terminal body weight. In addition, at 20000 ppm, $F_1$ male and female parents had significantly increased relative liver weights (11.4% and 28.3%, respectively) compared to controls. There was also a treatment-related increase in the incidence of bile duct proliferation in $F_1$ parental males at 20000 ppm. There were no treatment-related effects on reproductive parameters in $F_0$ and $F_1$ parental animals of both sexes (sperm parameters in males and estrus cycles, pre or post-implantation losses in females), at all dose levels.

Among the offspring, there were no treatment-related effects on the number of pups born, live birth index, pups sex ratio, mean litter size at birth in $F_1$ or in $F_2$ pups at all dose levels. At 20000 ppm, slightly reduced litter weights were observed at weaning in both generations, and were considered toxicologically significant. In addition, $F_1$ pups at 20000 ppm group, had a significantly lower mean lactation index than the controls. Among $F_2$ pups, there was no treatment related change in the lactation indices at all dose levels. There were no treatment-related malformations, skeletal deviations, maturation of external sexual

organs, or gross pathological findings in any of the $F_1$ or $F_2$ pups at all dose levels,. The relative liver weights of weanling $F_2$ male and females at 20000 ppm were significantly higher (13 to 15% ) than the controls. The mean weights of other organ systems of treated animals did not differ from the controls.

The LOAEL for parental systemic toxicity was 20000 ppm (2509 mg/kg bw), based on decreased body weights ($F_1$ males), increased relative liver weights in both sexes and bile duct proliferation in $F_0$ and $F_1$ parental males. The NOEL for parental systemic toxicity was 2000 ppm (214.9 mg/kg bw/day in females). There were no effects on fertility or reproductive performance at all dose levels.

The LOAEL for reproductive/developmental toxicity was 20000 ppm (2509 mg/kg bw), based on deceased mean litter weight at day 28, ($F_1$ and $F_2$), reduced body weight development in $F_1$ and $F_2$ pups during lactation, increased pup relative liver weights and reduced lactation index in $F_1$ pups at 20000 ppm. The NOEL for reproductive/developmental toxicity was 2000 ppm (214.9 mg/kg bw/day in females).

The reproductive study in the rats is classified as acceptable. It satisfies the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800, §83-4); OECD 416 in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided

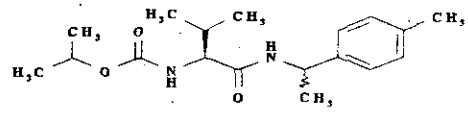
# I. MATERIALS AND METHODS

## A. MATERIALS:

1 **Test Material:** SZX 0722

| | |
|---|---|
| **Description:** | Technical, white powder |
| **Lot/Batch #:** | 05013/0212 |
| **Purity:** | 98.7 % a.i. |
| **Compound Stability:** | Stable at room temperature |
| **CAS #:** | 140923-17-7 |

Structure



2. **Vehicle and/or positive control:** 1% peanut oil (DAB 10) Lot/Batch # , Purity; not provided

3 **Test animals:**

| | | |
|---|---|---|
| **Species:** | Rat | |
| **Strain:** | ICO:WU (IOPS-Cpb | |
| **Age at study initiation:** | 6-7 weeks old | |
| **Wt. at study initiation:** | 104-140g (males), 76.4-131g (females) | |
| **Source:** | IFFA Credo/France, Belgium | |
| **Housing:** | Group caged by sex in Type II makrolon during acclimatization, and individually caged in Type III Makrolon cages during mating | |
| **Diet:** | Altromin rat maintenance diet, # 1321 (Altromin, GmbH, Lage), fed *ad libitum* | |
| **Water:** | Tap water in polycarbonate bottles | |
| **Environmental conditions:** | **Temperature:** | 23 ± 2°C |
| | **Humidity:** | 55 ± 5% |
| | **Air changes:** | 15-20/h |
| | **Photo period:** | 12h light/12h dark |
| **Acclimation period:** | 7 days | |

## B. PROCEDURES AND STUDY DESIGN

### 1. Mating procedure:

One male was caged with 1 female from the same test group until sperm cells were observed in vaginal smears taken daily during the mating period. If sperm were not found after 7-12 days of observation, the first male was removed and replaced by another male with proven fertility in the same test group. If two attempts at mating were unsuccessful, no further matings were tried. Sibling matings was avoided for $F_1$. After successful mating, each pregnant female was individually placed into a cage with a solid bottom and bedding where it was kept throughout the gestation and lactation periods.

2. Study schedule:
Parental ($F_0$) animals were pre-treated with the test substance for 10 weeks before and during pairing and throughout the gestation and lactation periods of the $F_1$ generation. Four days post-partum, the $F_1$ litters were culled to 8 pups which were raised and weaned at day 28 post partum. Thirty male and 30 female $F_1$ rats per group were selected for further treatment to breed the $F_2$ generation. The remaining pups weanlings were necropsied. The $F_0$ females were killed and necropsied after weaning of $F_1$ pups. The $F_0$ males were killed in the course of spermatological investigations performed about one month later. The selected $F_1$ offspring were treated (from weaning at 4 weeks) with the compound up to the age of at least 16 weeks, and then co-housed for mating. The $F_1$ parent animals were killed as scheduled after their $F_2$ litters had been weaned at day 28 postpartum.

All animals were weighed at the start of the study. Males were weighed weekly up to week 20, and the females until week 10 (end of pre-mating period). After insemination had been established, females were weighed on post-coital days 0, 7, 14, and 20; and on days 0, 4, 7, 14, 21, and 28 after birth of their pups. $F_0$ and $F_1$ animals were weighed on the date of necropsy to permit calculation of relative organ weights. Ten male and 10 female $F_2$ weanlings were further treated with the compound and used to determine the age at which praeputial separation or vaginal opening occurred. The rats were housed singly, and the praeputium and vagina were investigated on days 28, 31, 34, 37, and 40.

3. Animal assignment: Parental animals were assigned to test groups as shown in Table 1, using computer derived random numbers

TABLE 1 Animal Assignment

| Test Group | Dose in Diet[a] ppm | Animals/group | | | |
|---|---|---|---|---|---|
| | | $F_0$ Males | $F_0$ Females | $F_1$ Males | $F_1$ Females |
| Control | 0 | 30 | 30 | 28 | 28 |
| Low (LDT) | 100 | 30 | 30 | 29 | 29 |
| Mid (MDT) | 2000 | 30 | 30 | 28 | 28 |
| High (HDT) | 20000 | 30 | 30 | 30 | 30 |

a Diets were administered from beginning of the study until sacrifice.

4. Dose selection rationale: The doses were selected on the basis of a range finding, one generation reproduction study (T3059162) in which groups of 10 animals of either sex per dose were fed diets containing 0, 500 and 20000 ppm of the test material during a 4 week pre-mating period and thereafter . As the $F_0$ parents at 20000 ppm had higher relative liver weights, but no treatment related effects on reproductive parameters, that dose was used as the highest dose in this study.

5. Dosage preparation and analysis

Feed formulations were prepared immediately prior to study initiation, and stored at 5°C. SZX 0722 was blended with Altromin® 1321 containing 1% peanut oil to minimize dust formation (including 0 ppm concentration). The amounts of test substance were calculated on the basis of an assumed 100% content of SZX 0722. Homogeneity and stability of the test compound in diet preparations were done prior to commencement of the study, using sample mixtures. Results showed even distribution in the diet amount used, and stability in the concentration range used throughout the feeding period (1 week). Test substance content was checked at regular intervals throughout the study (start of the study, randomly each 3 month period, and at the end of the study.

**Results - Homogeneity Analysis:** Even distribution 97.5 % of nominal values.

**Stability Analysis:** 90.5-103.1% of nominal value, stable for 14 days in feed at room temperature.

**Concentration Analysis:** 98.7 -99.0 of nominal value

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

## C. OBSERVATIONS

1. Parental animals:

Observations and the schedule for those observations are summarized from the report. They included mortality and clinical signs, detailed examinations, body weight and food consumption, sperm parameters and sexual maturation. Feed intake of males was measured weekly except during the mating period. In females, feed intake determinations were performed weekly during pre-mating and on post-coital days 7, 14, and 20 as well as on days 4 and 7 after delivery of the litter. Reproductive parameters investigated in females included: oestrus cycle staging, insemination index, fertility index, gestation index and duration of pregnancy.

2. Litter observations:

The following litter observations were made for both the $F_1/F_2$ litters: Number and sex of live and dead pups, were determined on postpartum day 0, day 4 (before and after reduction), 7, 14, 21, and 28. Body weights and clinical signs were also recorded at these time points. Any apparent malformations were noted. Insemination index, fertility index, gestation index, live birth index, viability and lactation index were all calculated for each dose. On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (as nearly as possible to 4/sex/litter). Excess pups were killed and discarded.

Dead pups were examined grossly for external and internal abnormalities. A possible cause of death was determined for pups born or found dead.

3. **Postmortem observations:**

1) **Parental animals:**
All surviving parental males were sacrificed as soon as possible after the last litters in each generation were produced. Maternal animals were sacrificed after the last litter of each generation was weaned.
Parents that died or were killed in moribund condition during the study were necropsied and examined macroscopically. After the $F_1$ and $F_2$ pups had been weaned, the scheduled necropsies of the dams were conducted. In $F_0$ and $F_1$ females, implantation sites were counted.

Tissue samples from the reproductive related organs (including coagulation glands, epididymis, mammary glands, ovaries, oviducts, pituitary gland, prostate gland, seminal vesicles, skin, testes, uterus including cervix, and vagina) of the control and high dose groups of the $F_0$ and $F_1$ generations were processed examined microscopically. The liver and gross lesions were examined from all parent animals of all groups.

2) **Offspring:**
The $F_1$ offspring not selected as parental animals, and all $F_2$ offspring were sacrificed at 28 days of age. These animals were subjected to postmortem examinations (macroscopic and/or microscopic examination.

## D. DATA ANALYSIS

1. **Statistical analyses:**
Measure of central tendency and percentages were used to describe parametric data. The Dunnett's test with the variance analysis were used to analyse body weight and organ weights of parental animals. The Kruskal Wallis and Steel Test used to assess food consumption data. The U-Test was used to evaluate pup weights, litter sizes and weights. The Fisher's exact probability (two tailed) test was used to compare treated with controls for significance of differences at levels of a = 5% and a= 1%, in the evaluation of the mating index, gestation index, live-birth index, viability index and lactation index. The application of these statistical methods was appropriate.

2. **Indices:**

**Reproductive indices**: The mating, fertility and gestation indices, were calculated from breeding and parturition records of animals in the study.

**Offspring viability indices**: The viability indices at day- 0, 4, 7, 14, 21 and 28 and the lactation indices

were calculated from lactation records of litters in the study:

3. **Historical control data:** for the pertinent prenatal and offspring data were provided

## II. RESULTS

## A. PARENTAL ANIMALS

### 1. Mortality and clinical signs:

There were no treatment-related effects on the appearance and behaviour of $F_0$ males or females or $F_1$ animals at dose levels of up to 20000 ppm. In $F_0$ animals, one pregnant 100 ppm female was sacrificed in a moribund state, due to dystocia. Six $F_1$ animals (2 females at 0 ppm, 1 female at 100 ppm, and 1 male 2 and females at 2000 ppm) were sacrificed in *extremis*, due to severe inflammation of skin and eyes. These severe skin and eye inflammatory conditions were considered incidental as they occurred in both the controls and treated groups in a non-dose-related manner, although none occurred at 20000 ppm. Therefore, there was no treatment-related mortality in $F_0$ males or females or $F_1$ animals at levels of up to 20000 ppm.

### 2. Body weight and food consumption:

The body weights of the male and female $F_0$ or $F_1$ animals up to the 2000 ppm dose level did not differ from those of controls (Figures 1 and 2). In the 20000 ppm group, males of the $F_0$ generation had slightly reduced body weights (maximally 6%, $p \leq 0.05$) from week 13 onwards. The $F_1$ generation males had significantly lower body weights (approx. 10% in week 13), which was considered toxicologically significant. In 20000 ppm females of the $F_0$ generation, slight to moderate body weight depression, was detected during the pre-mating (< 5%) and lactation (up to 7%) periods (Table 2) In the $F_1$ females, the body weight reduction reached statistical significance only during week 13 and on lactation day 14, and were not considered toxicologically significant.

Figure 1: Body weights. F0 Parental animals

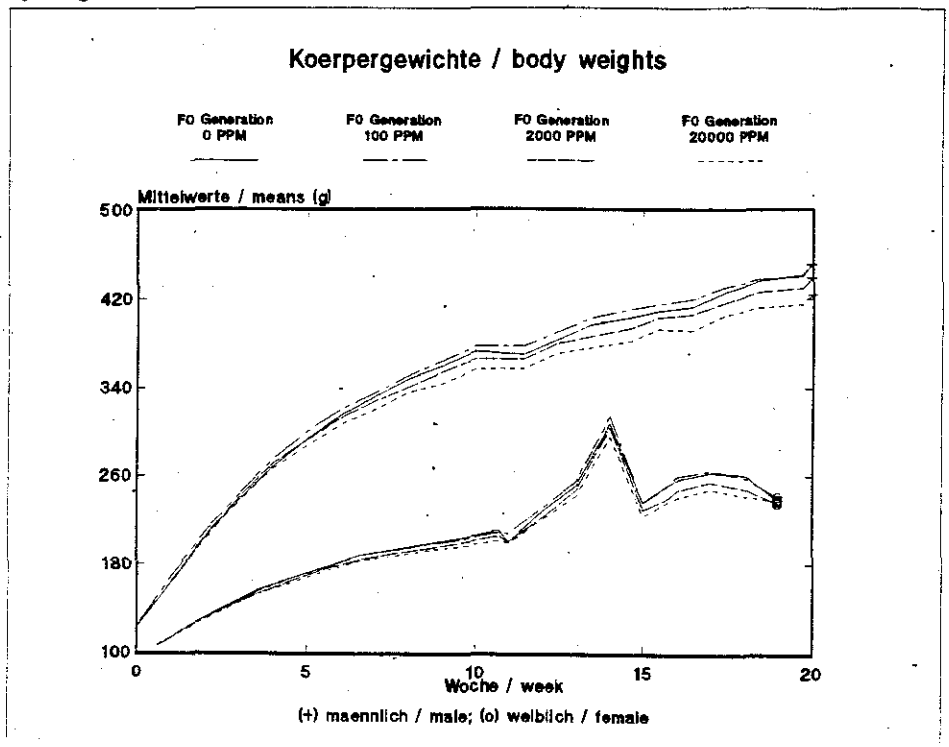


Figure 2.Figure 1: Body weights, F1 Parental animals

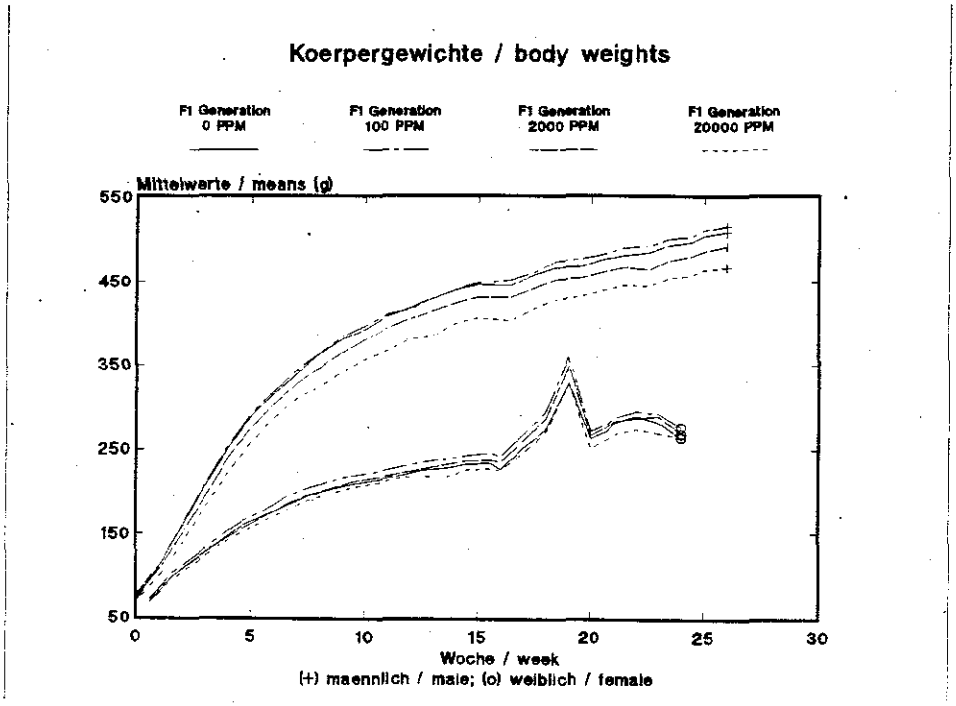


**Table 2. Body weights of females during lactation**

| | Female body weight (g) on lactation day | | | | | |
|---|---|---|---|---|---|---|
| **Lactation day** | **0** | **4** | **7** | **14** | **21** | **28** |
| **Dose level (ppm)** | | | | | | |
| **0** $F_0$ | 237 | 252 | 257 | 263 | 262 | 239 |
| $F_1$ | 265 | 272 | 282 | 290 | 283 | 265 |
| **100** $F_0$ | 236 | 251 | 260 | 265 | 260 | 242 |
| $F_1$ | 273 | 281 | 288 | 295 | 293 | 277 |
| **2000** $F_0$ | 230 | 239 | 248 | 255 | 249 | 236 |
| $F_1$ | 268 | 278 | 284 | 288 | 290 | 269 |
| **20000** $F_0$ | 224 | 236$^+$ | 241$^+$ | 249 | 243$^+$ | 238 |
| $F_1$ | 251 | 263 | 269 | 275$^+$ | 271 | 265 |

U-test: $^+$ = 5% significance level

**Food consumption**

In $F_0$ and $F_1$ parents during pre-mating, gestation and lactation, the feed intake per animal and per day did not differ from the controls to a toxicologically relevant extent in treated males and females up to 2000 ppm (Table 3). In the 20000 ppm dose group, $F_0$ and $F_1$ females consumed 14.7% and 23% per kg bw, respectively, more feed than controls during the pre-mating period.

Table 3. Two-generation study in rats: Mean Food consumption (g/kg bw/day),of females pre-mating

| **Dose** | **0 ppm** | **100 ppm** | **2000 ppm** | **20000 ppm** |
|---|---|---|---|---|
| **Females** | | | | |
| $F_0$-generation | 90.4 | 96.2 | 95.2 | 103.7 |
| $F_1$-generation | 119.6 | 107.9 | 119.7 | 147.2 |

**3. Test Substance Intake:**

Based on food consumption, body weight, and dietary analyses results, the doses expressed as mean daily mg test substance/kg body weight during the pre-mating period are presented in Table 4. The values for the $F_0$ or $F_1$ generation are considered to be representative of the test substance intake for the entire study.

**TABLE 4:** Mean daily test substance intake during pre-mating (mg/kg body weight/day)[a]

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | **100** | **2000** | **2000** | **100** | **2000** | **20000** |
| $F_0$ | 7.3 | 146.3 | 1514.3 | 9.6 | 190.4 | 2074 |
| $F_1$ | 7.7 | 155.3 | 1838 | 10.8 | 239.5 | 2944.1 |
| Mean | 7.5 | 150.8 | 1676.2 | 10.1 | 214.95 | 2509.05 |

a Data extracted from pg 39 of the study report.

**4. Reproductive function:**

**a. Estrous cycle length and periodicity:**

There were no treatment related effects on estrous cycles or cyclicity of animals.

**b. Sperm measures:**

Results from the evaluation of sperm parameters revealed no treatment related effects on sperm morphology or motility. In $F_0$ males, a slight reduction in initial (at 1 minute) sperm motility was detected at 20000 ppm group. However, 14 of 15 males achieved fertilization with their females. There was no dose-related effect on sperm motility. Some sperm abnormalities (head-tail break, head-tail separation or sharps in the tail) were found more frequently at 20000 ppm than in other groups. However, this was attributable to a high incidence of head-tail separation in one particular animal. In $F_1$ males, no effect was detected on any sperm parameters at 20000 ppm. Consequently, males of the 100 and 2000 ppm were not examined in this respect.

**c. Sexual maturation ($F_1$):**

There were no treatment related effects on the sexual maturation at any of the dose level.

**5. Reproductive performance:**

Results of reproductive performance for the parental animals are summarized from the report in Table 5. The insemination (mating), fertility and gestation indices of all treated animals did not differ in a toxicologically relevant manner from the pertinent controls in either generation.

TABLE 5: Reproductive Performance of $F_0$ and $F_1$ parental animals

| Observation | Dose Group (ppm) | | | |
|---|---|---|---|---|
| | 0 | 100 | 2000 | 20000 |
| **$F_1$ Generation - Litter 1** | | | | |
| **MALES** | | | | |
| Mated, $F_0$ | 30 | 30 | 30 | 30 |
| Mated, $F_1$ | 28 | 29 | 28 | 30 |
| **FEMALES** | | | | |
| Number mated, $F_0$ | 30 | 30 | 30 | 30 |
| Number mated, $F_1$ | 28 | 29 | 28 | 30 |
| Insemination (mating) index (%), $F_0$ | 100 | 100 | 100 | 100 |
| Insemination (mating) index (%), $F_1$ | 100 | 100 | 96.4 | 93.1 |
| Fertility index (%), $F_0$ | 76.7 | 73.3 | 80 | 76.7 |
| Fertility index (%) $F_1$, | 78.6 | 93.1 | 88.8 | 96.4 |
| Gestation index (%), $F_0$ | 100 | 95.4[b] | 100 | 100 |
| Gestation index (%), $F_1$ | 100 | 100 | 100 | 100 |
| Gestation interval (days), $F_0$ | 22.4 | 22.2 | 22.2 | 22 |
| Gestation interval (days), $F_1$ | 22.4 | 22.2 | 22.2 | 22. |
| Number of litters, $F_0$ | 23 | 21[b] | 24 | 23 |
| Number of litters, $F_1$ | 23 | 20 | 24 | 23 |

a Data extracted from pgs 44 and 58 of the study report.; [b] one female had dystocia

## 6. Parental postmortem results

**a) Organ weights:** In the $F_0$ parents, the liver and testes weights were comparable with the controls up to 2000 ppm. At 20000 ppm, higher relative liver weights were observed in males (14%) and in females (22.2%), and there was a significant increase (10%) in the relative testes weights (Table 6). Among $F_1$ parents, the absolute liver weights (males only) and relative liver weights were significantly elevated in males (11.4% ) and females (28.3%). The increase in liver weight was considered toxicologically significant, as it was associated with microscopic changes in the tissue. The slight increase in relative testes weights of 20000 ppm $F_0$ rats were not considered adverse effects because there were no accompanying morphologic alterations in that tissue, and testis weights of treated $F_1$ males were unaffected in all dose levels.

**Table 6.** Absolute and relative liver weights

| Sex | | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Dose level (ppm)** | | **0** | **100** | **2000** | **20000** | **0** | **100** | **2000** | **20000** |
| **Abs. liver wt. [mg]** | $F_0$ | 15133 | 15740 | 15023 | 16340$^{+}$ | 10048 | 9680 | 10204 | 12230$^{++}$ |
| | $F_1$ | 17874 | 18601 | 17351 | 18521 | 10481 | 10677 | 11294 | 13363$^{++}$ |
| **Rel. liver wt. [mg/100 g bw]** | $F_0$ | 3265 | 3338 | 3285 | 3713$^{++}$ | 4206 | 4025 | 4252 | 5142$^{++}$ |
| | $F_1$ | 3422 | 3507 | 3353 | 3811$^{++}$ | 3936 | 3870 | 4187 | 5051$^{++}$ |
| **Rel. testes weights** (mg/100 g bw) | $F_0$ | 722 | 717 | 728 | 794$^{+}$ | - | - | - | - |

Dunnett-test: + = 5 % significance level, ++ = 1 % significance level

**b) Pathology**

**1) Macroscopic examination:**

There were no treatment-related gross pathological findings among male or female $F_0$ or $F_1$ animals at doses of up to 20000 ppm. In all dose groups, the number of implantation sites was comparable to the number of pups born, and did not differ from the controls. Therefore there was no treatment related pre- or post-implantation losses at any dose level.

**Microscopic examination:**

There were notable increases in the number of $F_1$ and $F_2$ parents with minimal to slight cytoplasmic changes in hepatocytes, at 2000 and 20000 ppm groups (Table 7). This change in hepatocytes staining characteristics was not considered to be of toxicological significance. There was a treatment-related increase in the incidence of bile duct proliferation in $F_1$ males at 20000 ppm, which was considered by the reviewer to be toxicologically significant. All findings in other tissues in both sexes, at all dose levels were within the normal historical control range, and were considered to be incidental.

Table 7. Histopathological findings.

| | No. of $F_0$ rats with treatment-related liver findings | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sex | Males (n = 30/group) | | | | Females (n = 30/group) | | | |
| Dose group | 0 | 100 | 2000 | 20000 | 0 | 100 | 2000 | 20000 |
| Minimal to slight cytoplasmic changes in hepatocytes | 0 | 0 | 22 | 30 | 0 | 0 | 15 | 29 |
| bile duct hyperplasia | 4 | 3 | 7 | 6 | 2 | 0 | 0 | 0 |
| biliary fibrosis | 5 | 2 | 4 | 2 | 0 | 0 | 0 | 0 |
| | No. of $F_1$ rats with treatment-related liver findings | | | | | | | |
| Minimal to slight cytoplasmic changes in hepatocytes | 0 | 0 | 2 | 28 | 0 | 0 | 10 | 26 |
| bile duct hyperplasia* | 5 | 3 | 4 | 13 | 0 | 0 | 0 | 0 |
| biliary fibrosis | 4 | 3 | 3 | 6 | 0 | 0 | 0 | 0 |

*Data from page 551.555, report 3,(Vol 21, DACO 4.5.1-2)

## B. OFFSPRING

### 1. Viability and clinical signs:

Results of pup parameters are summarized in Table 8 . There were no treatment related malformations and no effects on litter size in both generations. A slight shift in the sex ratio apparent at 2000 ppm and above but was within the historical control range, hence considered incidental. No significant clinical findings were observed in either the $F_1$ or $F_2$ pups during the four week lactation period at all dose levels. The mean viability indices of the treated groups were comparable to the the pertinent controls. The lactation of $F_1$ pups was not affected up to the dose of 2000 ppm, but dams in the 20000 ppm group showed a significantly lower mean lactation index, than the controls. The lactation indices of $F_2$ pups were not affected up to the dose of 20000 ppm. A relatively high incidence of cannibalism was reported among all $F_1$ animals (control and treated groups), but an explanation for this behaviour was not provided. There were no treatment related effects on the maturation of external sexual organs in all dose levels.

**Table 8.** Litter parameters for $F_1$ and $F_2$ litters [a]

| Observation | $F_1$ Generation | | | | $F_2$ Generation | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 100 | 2000 | 20000 | 0 | 100 | 2000 | 20000 |
| Total number of pups | 230 | 206 | 249 | 234 | 211 | 296 | 259 | 277 |
| Mean litter size | 9.68 | 10.1 | 10 | 9.95 | 9.54 | 10.37 | 10.54 | 10.22 |
| # Deaths Days 0-4 (%) | 3 | 5 | 9 | 5 | 1 | 16 | 6 | 1 |
| Live birth index | 98.9 | 97.8 | 96.6 | 97.8 | 99.5 | 95.0 | 97.7 | 99.7 |
| Males (%) | 49 | 49 | 53 | 53 | 50.2 | 52.4 | 52.5 | 53.8 |
| Females (%) | 51 | 51 | 47 | 47 | 49.8 | 47.6 | 47.5 | 47 |
| Viability index (days 4) | 98.9 | 93.4 | 96.1 | 94.4 | 88.9 | 89.8 | 88.6 | 86.4 |
| Lactation index, day- 21 | 84.4 | 95.4 | 86.0 | 66.5** | 58.2 | 55.9 | 75.0 | 56.4 |
| Lactation index , day -28 | 84.4 | 95.4 | 86.0 | 66.5** | 58.2 | 55.9 | 75.0 | 56.4 |

[a] Data extracted from pgs 49, 61, 63 of the study report.

### 2. Body weights of $F_1$ and $F_2$ pups.

In the $F_1$ generation, at 20000 ppm, there was a treatment related reduction (23.5%) in the mean litter weight at weaning (Table 9). In $F_2$ pups on day 28, litter weights at 20000 ppm were reduced by 13.5% compared to control, which though not statistically significant, was considered toxicologically significant
Individual pup birth weights and body weight gains of $F_1$ pups during lactation were unaffected up to 2000 ppm (Table 11). At 20000 ppm, slightly reduced mean pup birth weight and lower pup body weights (male pups sometimes $p \leq 0.05$) were recorded during lactation. Individual birth weights of $F_2$ pups were unaffected up to the 20000 ppm dose level. Mean body weights of male and female $F_2$ pups during lactation were unaffected up to 2000 ppm, but significantly lower body weights were noted in male ($p \leq 0.05$) and females pups ($p \leq 0.01$) at

20000 ppm, on day 28.

**Table 9.** Mean litter weights (g) at birth and at weaning

| | | 0 ppm | 100 ppm | 2000 ppm | 20000 ppm |
|---|---|---|---|---|---|
| **Day 0 (at birth)** | $F_1$ | 59.40 | 60.19 | 60.50 | 57.66 |
| | $F_2$ | 60.70 | 62.09 | 65.60 | 61.32 |
| **Day 28** | $F_1$ | 446.71 | 531.40** | 462.39 | 342.18* |
| | $F_2$ | 328.37 | 291.61 | 342.70 | 283.79 |

Statistically different from control,* = $p \leq 0.05$ , ** $p \leq 0.01$

**Table 10.** Mean body weights of pups during lactation (g)

| | | 0 ppm | | 100 ppm | | 2000 ppm | | 20000 ppm | |
|---|---|---|---|---|---|---|---|---|---|
| | | $F_1$ | $F_2$ | $F_1$ | $F_2$ | $F_1$ | $F_2$ | $F_1$ | $F_2$ |
| Days after culling | | | | | | | | | |
| 4 | m | 9.50 | 9.40 | 9.98 | 8.63 | 9.50 | 8.95 | 8.80 | 8.94 |
| | f | 9.35 | 8.83 | 9.85 | 8.56 | 9.14 | 8.54 | 8.84 | 8.31 |
| 7 | m | 14.20 | 12.68 | 15.79** | 11.90 | 14.14 | 11.85 | 12.36* | 11.66 |
| | f | 13.59 | 11.82 | 15.30* | 12.08 | 13.79 | 11.34 | 12.38 | 10.88 |
| 14 | m | 28.55 | 26.42 | 29.91 | 28.26 | 28.93 | 25.58 | 27.17 | 25.94 |
| | f | 26.89 | 24.51 | 29.16 | 26.88 | 28.21 | 27.80 | 26.69 | 23.86 |
| 21 | m | 46.10 | 45.03 | 47.45 | 47.38 | 45.23 | 43.70 | 41.61* | 41.75 |
| | f | 43.64 | 43.00 | 45.95 | 44.33 | 44.76 | 45.45 | 40.52 | 38.76 |
| 28 | m | 72.94 | 73.17 | 74.57 | 75.44 | 72.25 | 69.09 | 64.68** | 66.59* |
| | f | 66.10 | 67.44 | 68.99 | 67.82 | 68.40 | 68.54 | 61.23 | 59.41** |

Statistically different from control,* = $p \leq 0.05$ , ** $p \leq 0.01$

**3. Offspring postmortem results:**

**a) Organ weights of $F_2$ weanlings:**

The mean weights of the brain, spleen, thymus, testes and ovaries showed no notable difference between the controls and treatment groups. The liver weights of weanlings showed no significant difference up to 2000 ppm. Male and female $F_2$ weanlings receiving 20000 ppm had 13 to 15% higher relative liver weights compared to controls (Table 11).

**Table 11.** Relative liver weights (mg/100g bw) of $F_2$ weanlings (no statistical evaluation)

| Dose | 0 ppm | 100 ppm | 2000 ppm | 20000 ppm |
|---|---|---|---|---|
| **Males** | 4442 | 4331 | 4565 | 5127 |
| **Females** | 4278 | 4249 | 4522 | 4837 |

b) Pathology

1) Gross pathological changes in $F_1$ and $F_2$ pups or weanlings

No treatment-related macroscopic alterations or skeletal deviations were observed in a $F_1$ or $F_2$ pups up to the 20000 ppm dose levels. No gross pathological findings were reported in either $F_1$ or $F_2$ weanlings at scheduled necropsy. The mean time of preputial separation and vaginal opening in all dose levels was comparable to the controls.

2) Microscopic examination:

Microscopic examination of weanling rat tissues was not performed.

## III. DISCUSSION

A. Investigators' conclusions:

The LOAEL for parental systemic toxicity was 20000 ppm (2509 mg/kg bw/day in females), based on reductions in body weight of males and increases in liver weights, which were accompanied by histopathologic changes in the livers of both sexes at that dose. The NOAEL for parental systemic toxicity was 2000 ppm (214.9 mg/kg bw/day in females).

The LOEL for reproductive/developmental toxicity was 20000 ppm (2509 mg/kg bw/day in females), based on retarded body weight development in $F_1$ and $F_2$ pups during lactation, slightly reduced mean litter weight at birth and at day 28, and increased relative pup liver weights, and a reduced lactation index in $F_1$ pups at 20000 ppm The NOEL for reproductive/developmental toxicity was 2000 ppm (214.9 mg/kg bw/day in females).

B. Reviewer's discussion:

I concur with the study author's conclusions. The NOEL for parental systemic toxicity was 2000 ppm (214.9 mg/kg bw/day in females). There were no effects on fertility or reproductive performance at the highest dose (2509 mg/kg bw/day). The NOEL for reproductive/developmental toxicity was 2000 ppm (214.9 mg/kg bw/day in females).

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The reproductive study in the rats is classified acceptable and satisfies the guideline requirement for a 2 -generation reproductive study (OPPTS 870.3800, §83-4); OECD 416 in the rat.

C. Study deficiencies: There were no study deficiencies that would affect the acceptability of this study.

**IPROVALICARB**

Two-generation dietary reproduction study in rats: **MRID No. 44865720**

Body Weights for $F_0$ and $F_1$ Generation Adults
(Refer to body weights DER pages 8-10)

| Body weights in grams | | 0 ppm | | 100 ppm | | 2000 ppm | | 20000 ppm | |
|---|---|---|---|---|---|---|---|---|---|
| | | $F_0$ | $F_1$ | $F_0$ | $F_1$ | $F_0$ | $F_1$ | $F_0$ | $F_1$ |
| $F_0$ | $F_1$ | MALES | | | | | | | |
| week 0 | week 0 | 123 | 71 | 125 | 78 | 123 | 75 | 124 | 70 |
| week 1 | week 1 | 164 | 111 | 169 | 113 | 165 | 109 | 163 | 101 |
| week 10 | week 13 | 373 | 430 | 378 | 431 | 366 | 415 | 357 | 385** |
| week 20 | week 26 | 451 | 509 | 451 | 517 | 439 | 492 | 424 | 467* |
| bw gain 0-20 or 26 | | 328 | 438 | 326 | 439 | 316 | 417 | 300 | 397 |
| PREMATING | | FEMALES | | | | | | | |
| week 0 | week 0 | 106 | 69 | 106 | 72 | 106 | 69 | 107 | 67 |
| week 1 | week 1 | 124 | 99 | 125 | 103 | 124 | 99 | 123 | 95 |
| week 10 | week 15 | 211 | 233 | 209 | 246 | 206 | 237 | 202 | 227 |
| bw gain 0-10 or 15 | | 105 | 164 | 103 | 174 | 100 | 168 | 95 | 160 |
| GESTATION | | FEMALES | | | | | | | |
| day 0 | day 0 | 200 | 226 | 208 | 242 | 201 | 235 | 199 | 225 |
| day 20 | day 20 | 308 | 329 | 315 | 360 | 305 | 349 | 296 | 329 |
| body wt gain (0-20) | | 108 | 103 | 107 | 118 | 104 | 114 | 97 | 104 |
| LACTATION | | FEMALES | | | | | | | |
| day 0 | day 0 | 237 | 265 | 236 | 273 | 230 | 268 | 224 | 251 |
| day 7 | day 7 | 257 | 282 | 260 | 288 | 248 | 284 | 241 | 269 |
| day 14 | day 14 | 263 | 290 | 265 | 295 | 255 | 288 | 249 | 275 |
| day 21 | day 21 | 262 | 283 | 260 | 293 | 249 | 290 | 243* | 238 |
| day 28 | day 28 | 239 | 265 | 242 | 277 | 236 | 269 | 238 | 265 |
| body wt gain (0-28) | | 2 | 0 | 6 | 4 | 6 | 1 | 14 | 14 |

p= :* = 0.05; ** = 0.01 [28-30 rats/sex/group]
Data extracted from Report pages 112-115 and 142-145; also, from DER page 10.